Effect of Neem Seed Extract on the Brown Citrus Aphid (Homoptera: Aphididae) and its Parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae)

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ABSTRACT The biological effects of a commercially available neem seed extract (Neemix 4.5, 4.5% azadirachtin, AZ) were assessed on the brown citrus aphid, *Toxoptera citricida* (Kirklady), a recently introduced insect pest of citrus in the United States and its parasitoid, *Lysiphlebus testaceipes.* When small citrus seedlings were dipped with the neem extract at 11–180 ppm AZ, 0–8% of nymphs and 0–17.5% of adults survived 7 d after the treatment while 95% of nymphs and 42.5% of adults in the control survived for the same period. The extract drastically reduced longevity of both adults and nymphs, adult fecundity, and molting of nymphs at all tested concentrations. Spraying neem extract (11–180 ppm AZ) onto potted citrus plants in the greenhouse also significantly reduced aphids by 20–100%, while control aphid populations increased by 950% 7 d after treatment. Application of the extract had little impact on the survival of adult parasitoids and developing parasitoids within aphids because parasite emergences were similar between treated and untreated parasitized aphids. These results indicate that neem extract may be compatible with integrated pest management programs in citrus and should be evaluated for field efficacy.

KEY WORDS *Toxoptera citricida, Lysiphlebus testaceipes,* neem extract, brown citrus aphid, Neemix, azadirachtin

In recent years, interest in developing natural insecticides has increased because of drawbacks in the use of synthetic insecticides. For example, environmental pollution, development of insecticide resistance, insecticide-induced resurgence of insect pests, and adverse effects on nontarget organisms have been problems. One natural insecticide of interest is an extract from seeds of the neem tree (*Azadirachta indica* A. Juss.) which can be efficacious and is biodegradable (Isman 1999). The primary active ingredient of most neem-based pesticides is azadirachtin (AZ), a liminoid compound with excellent insecticidal activity against many phytophagous pests. Azadirachtin has numerous effects on insect pests, including insect growth regulation (IGR), feeding deterrence, and reproduction inhibition (Mordue et al. 1998, Walter 1999). Equally important, neem extract has minimal toxicity to nontarget organisms such as parasitoids, predators, and pollinators (Lowery and Isman 1995, Naumann and Isman 1996) and degrades rapidly in the environment (Isman 1999).

The biological activities of neem extract (or its most active constituent, azadirachtin) are known for >400 insect pests (Schmutterer and Singh 1995). However, there have been few detailed studies on the effects of neem extract on citrus insect pests and their natural enemies. The brown citrus aphid, *Toxoptera citricida* (Kirkaldy), is a newly introduced pest of citrus in the United States. The brown citrus aphid spread through Central America and the Caribbean islands during the late 1980s (Yokomi et al. 1994), and was first detected in southern Florida in November 1995 (Halbert and Brown 1996). Since then, the aphid has spread to many citrus-growing areas in south and central Florida (Halbert 1997) and is a major concern to citrus growers throughout the United States because of its reportedly great efficiency in transmitting certain strains of citrus tristeza virus (CTV) (Yokomi et al. 1994). Because of the threat this pest poses, we investigated a commercially available neem extract (Neemix 4.5, Thermo Trilogy, Columbia, MD) for its biological activities, including effects on survival, longevity, reproduction, and development, against the brown citrus aphid (*T. citricida)* and the native parasitoid *Lysiphlebus testaceipes* (Cresson).

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Table 1. Survival, longevity, and reproduction of brown citrus aphid adults exposed to small citrus seedlings treated with Neemix

Treatment (AZ level), ppm	% survival after treatment				Longevity (days)	Avg. no. offspring[a]
	1 d	2 d	4 d	7d		
Control	97.5a	97.5a	90.0a	42.5a	5.7a	17.1a
11	97.5a	92.5ab	62.5b	17.5b	4.3b	6.0b
22	95.0a	90.0ab	50.0bc	10.0b	4.0b	5.4bc
45	95.0a	92.5ab	57.2bc	5.0b	3.5c	4.6bc
90	95.0a	82.5bc	32.5cd	2.5b	3.1cd	3.3bc
180	95.0a	77.5c	17.5d	0.0b	2.9d	2.5c

Each treatment was replicated eight times (i.e., eight small citrus seedlings) and five adult aphids were transferred to each treated seedling. Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).
[a] Total offspring per aphid during 7-d observation period.

Materials and Methods

Insect Source and Rearing. Laboratory colonies of the brown citrus aphid were established with field-collected aphids from grapefruit trees (*Citrus paradisi* Macf.) in a citrus grove near Orlando, FL. Parasitoids (*L. testaceipes*) were originally collected from a colony of brown citrus aphids on a potted citrus plant at the U.S. Horticultural Research Laboratory, Orlando, FL in November 1999. Colonies of aphids and parasitoids were maintained on potted sour orange (*Citrus aurantium* L.) seedlings (20–40 cm tall) in screened cages measuring 45 by 45 by 70 cm as described by Tang et al. (1999). Voucher specimens were deposited in the Florida State Collection of Arthropods at Gainesville, FL.

Chemicals. Neemix 4.5 (4.5% AZ) was obtained from Thermo Trilogy, Columbia, MD. The product was screened at concentrations of 0.4, 0.2, 0.1, 0.05, and 0.025% vol:vol (i.e., 180, 90, 45, 22, and 11 ppm AZ, diluted with distilled water) to evaluate toxic, reproductive, and developmental effects on the aphid and its parasitoid. Bioassays on the parasitoid did not include the 90 and 22 ppm AZ rates.

Laboratory Bioassays. Sour orange seedlings were grown to 1.5–2.5 cm tall in Rootcubes Growing Media (Smithers-Oasis, OH). The aerial portion of each seedling was dipped for five s in suspensions of Neemix, prepared at the concentrations described above, and then dried by placing the seedlings in a chemical hood for 1 h. Control seedlings were dipped similarly in distilled water. Separate experiments were conducted for aphid adults and nymphs. Groups of five adult aphids or ten second-instar nymphs were transferred discretely to each treated citrus seedling. Each seedling with the aphids was placed in a petri dish (9 cm diameter by 3 cm deep) in an incubator at 25 ± 1°C, 60–80% RH, and a photoperiod of 14:10 (L:D) h. Mortality of both adults and nymphs, numbers of offspring from adults, and numbers of molts by nymphs were recorded daily for 7 d. Percent survival and longevity of adults and nymphs, average offspring per adult, and average molts per nymph were calculated for each citrus seedling. Each treatment was replicated eight times.

Toxic effects of neem extract to *L. testaceipes* were determined by confining 10 pairs of the adult parasitoids (4–8 h after emergence) in a glass vial (1 by 7 cm) containing a citrus leaf dipped in suspensions of Neemix for five s and then dried for 1 h. Mortality of parasitoids was assessed at 8, 24, and 48 h postexposure. The emergence rate of adult parasitoids developed within mummified hosts (5–6 d after parasitism) was determined by dipping sections of sour orange foliage containing 50–60 aphid mummies in the Neemix suspensions. The number of emerged parasitoids in each treatment was recorded 7 d postexposure. Treatments for both tests were replicated six times.

Greenhouse Trials. Ten adult aphids were transferred to each of 120 potted sour orange seedlings (4 mo old and 20–25 cm tall) and allowed to produce offspring for 5 d before treatments were applied. Applications were via a hand-held sprayer and the plants containing aphids were sprayed until runoff. Numbers of live aphids (both adults and nymphs) per plant

Table 2. Survival, longevity, and development of the second-instar nymphs of brown citrus aphids exposed to the small citrus seedlings treated with Neemix

Treatment (AZ level), ppm	% survival after treatment				Longevity (days)	Avg no. Molts[a]
	1 d	2 d	4 d	7 d		
Control	100.0a	100.0a	97.5a	95.0a	6.7a	2.9a
11	93.5a	60.0b	33.0b	8.0b	3.1b	0.7b
22	90.0a	58.0b	30.0b	5.0b	2.9bc	0.4c
45	95.0a	51.5b	28.0b	0.0b	2.8bc	0.4c
90	92.5a	50.0b	15.0bc	0.0b	2.4cd	0.4c
180	90.0a	28.0c	8.0c	0.0b	2.2d	0.4c

Each treatment was replicated eight times (i.e., eight small citrus seedlings) and 10 second-instar nymphs were transferred to each treated seedling. Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).
[a] Total molts (exuviae) per aphid during 7-d observation period.

Table 3. Brown citrus aphid populations before and after foliar applications of Neemix on potted citrus plants in the greenhouse

Treatment (AZ level), ppm	Mean no. aphids per plant (n = 20)			Population increase index[a]
	Pre-test	2d Post-test	7d Post-test	
Control	47.9a	130.3a	447.8a	8.35
11	47.2a	35.8b	34.6b	−0.27
22	48.8a	31.8b	14.8c	−0.70
45	45.5a	23.3c	5.7c	−0.87
90	47.4a	11.3cd	2.7c	−0.94
180	57.0a	2.2d	0.0c	−1.00

Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).

[a] Population increase index = (no. aphids per plant 7d after treatment − no. aphids per plant before treatment) / (no. aphids per plant before treatment).

were recorded before the application, and at 2 and 7 d postapplication. An index of aphid population increase was calculated for each treatment as follows:

$$PI = (N_{post} - N_{pre}) / (N_{pre}),$$

where PI was the index of population increase, N_{pre} was the number of aphids per plant before treatment, and N_{post} was the number of aphids per plant 7 d after treatment. There were 20 potted citrus plants per treatment, and the entire trial was conducted within a small greenhouse beginning on 4 May 2000.

Data Analyses and Statistics. Data for aphid population density, survival, longevity, development, and offspring production, and for parasitoid emergence and survival were subjected to analysis of variance (ANOVA) and treatment differences were determined by Tukey's studentized range test. Differences among means were considered significant at a probability level of five percent ($P \leq 0.05$). Statistical tests were performed using PROC GLM (SAS Institute 1990). Probit analyses were conducted using the Probit Procedure (SAS Institute 1990) to generate LC_{50} values and slopes of probit lines for mortalities of aphid adults and nymphs due to treatments.

Results

Effects of Neem Extract on the Brown Citrus Aphid. In laboratory bioassays, the survival rates of adults ($F = 0.18$; df = 5, 47; $P = 0.97$) and nymphs ($F = 0.29$; df = 5, 47; $P = 0.29$) were not significantly different among all the tested concentrations (11–180 ppm AZ) and the controls 1 d after the test. However, the survival rates of treated nymphs at 2, 4 and 7 d postexposure were significantly different ($F = 3.9$; df = 5, 47; $P = 0.0074$; $F = 15.38$; df = 5, 47; $P < 0.0001$; $F = 8.65$; df = 5, 47; $P < 0.0001$; respectively) from those of control nymphs at all tested concentrations. Similarly, the survival rates of treated adults at 2, 4 and 7 d postexposure were significantly different ($F = 20.92$; df = 5, 47; $P < 0.0001$; $F = 61.80$; df = 5, 47; $P < 0.0001$; $F = 175.46$; df = 5, 47; $P < 0.0001$; respectively) from those of the controls at all concentrations except 11, 22, and 45 ppm at 2 d posttreatment (Tables 1 and 2).

The mortalities of adults and nymphs at 2 and 4 d after exposure to Neemix were dose-dependent. The LC_{50} values for adults were much higher than those for nymphs during the same periods. The LC_{50} values for adults at two and 4 d postexposure were 3782 ppm (95% FL = 472–3.17E18) and 30.37 ppm (95% FL = 16.52–46.78), respectively, and the slopes of the probit lines were 3.98 (SE = 1.84) ($\chi^2 = 4.84$, df = 1, $P = 0.0277$) and 9.12 (SE = 3.62) ($\chi^2 = 18.64$, df = 1, $P < 0.0001$). The LC_{50} values for nymphs were 41.91 ppm (95% FL = 24.30–69.58) and 3.99 ppm (95% FL = 0.61–8.66) and the slopes of the probit lines were 4.17 (SE = 1.22) ($\chi^2 = 16.82$, df = 1, $P < 0.0001$), and 5.75 (SE = 1.86) ($\chi^2 = 19.35$, df = 1, $P < 0.0001$), respectively, at 2 and 4 d postexposure. While 95% of nymphs and 42.5% of adults in the controls survived 7 d after the test, only 0–8% of nymphs and 0–17.5% of adults exposed to various concentrations of Neemix survived for the same period (Tables 1 and 2).

Neem extract drastically reduced longevity of adults and nymphs, adult fecundity (offspring), and nymphal development (molting) at all the tested concentrations (Tables 1 and 2). Means for these parameters were significantly different ($F = 82.53$; df = 5, 47; $P < 0.0001$ for adult longevity; $F = 51.99$; df = 5, 47; $P < 0.0001$ for adult offspring; $F = 106.35$; df = 5, 47; $P <$

Table 4. Effect of Neemix on adult survival and emergence rates of the parsitoid *L. testaceipes*

Treatment (AZ level), ppm	Percent survival after treatment			% adult emergence
	8 h	24 h	48 h	
Control	90.8a	79.2a	32.5a	85.5a
11	89.2a	80.0a	32.5a	83.3a
45	90.0a	74.3ab	33.3a	79.2ab
180	82.5b	69.2b	27.5a	73.5b

Means within a column sharing the same letter were not significantly different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1990]).

0.0001 for nymph longevity; and $F = 139.23$; df = 5, 47; $P < 0.0001$ for nymphal molts) from those of for the control group at all treatment concentrations. Production of offspring from treated adult aphids was 65–85% less than that of the control adults (Table 1). The aphid nymphs on treated citrus seedlings had less than one molt (0.4–0.7 molts on average), while the control nymphs molted ≈three times (2.9 molts on average) (Table 2).

In the greenhouse trials, the numbers of aphids per potted citrus plant were not significantly different ($F = 1.36$; df = 5, 119; $P = 0.25$) before treatments were applied. Spraying Neemix onto the plants resulted in significant reductions ($F = 125.93$; df = 5, 119; $P < 0.0001$; $F = 266.52$; df = 5, 119; $P < 0.0001$; respectively) in aphid populations per plant at 2 and 7 d posttreatment. The numbers of aphids per plant in the Neemix treatments were reduced 22–100% 7 d after application while the numbers of aphids per plant in the control group increased 950% during the same period (Table 3).

Effects of Neem Extract on *Lysiphlebus testaceipes*. Application of neem extract at 11 and 45 ppm AZ to citrus leaves did not significantly reduce the survival of adult parasitoids during the experiment. However, there was a small but significant reduction in survival caused by the 180 ppm treatment at 8 h ($F = 6.24$; df = 3, 23; $P = 0.0036$) and 24 h ($F = 4.45$; df = 3, 23; $P = 0.0150$) but not at 48 h posttreatment ($F = 1.56$; df = 3, 23; $P = 0.2291$) (Table 4). The emergence rates of parasitoids from mummified aphids treated with neem extract at 11 and 45 ppm AZ were not significantly different from the those of the control, while a small but significant decline in emergence was observed at 180 ppm AZ ($F = 6.43$; df = 3, 23; $P = 0.0032$) (Table 4).

Discussion

The brown citrus aphid is considered a serious insect pest to the citrus industry, not only because it develops enormous populations on flushing citrus, but also because it efficiently vectors CTV. Neem-based products have been observed to cause reductions in virus transmission by some species of aphids (Hunter and Ullman 1992, Mordue and Blackwell 1993). Effect of neem extract on CTV transmission and spread by the brown citrus aphid certainly deserves further examination.

Previous studies indicated that neem-based insecticides can be effective in controlling a number of aphid species (Lowery et al. 1993; Lowery and Isman, 1994; Schmutterer and Singh, 1995; Koul et al. 1997; Koul, 1998, 1999). However, there have been contradictory results regarding the toxicity to adult aphids. For instance, Lowery and Isman (1994) reported that the survival of nine species of adult aphids was unaffected by both the limonoid AZ and neem seed oil. In contrast, Koul (1999) indicated that neem extract was toxic to both nymph and adult aphids although the survival rate of treated adults was higher in comparison to treated nymphs. Walter (1999) suggested that different formulation components (other than AZ) of neem seed extracts are responsible for the variable toxicities and efficacies observed. Our laboratory and greenhouse evaluations of the biological effects of Neemix on the brown citrus aphid indicate that Neemix (11–180 ppm AZ) can significantly reduce survival rates and longevity of aphid adults and nymphs, as well as adult fecundity (offspring) and nymphal development (molting). However, the lethal concentration of AZ resulting in 50% mortality (LC_{50}) of adult aphids (3782 ppm at 2 d and 30.37 ppm at 4 d posttreatment) was much higher than that of aphid nymphs (41.91 ppm at 2 d and 3.99 ppm at 4 d posttreatment). Mortality of aphid adults and nymphs apparently was time-related (Tables 1 and 2). Consequently, toxicity of Neemix to the brown citrus aphid may be primarily due to feeding inhibition and/or IGR activity.

Neem-based insecticides have been found to have little impact on many beneficial organisms such as honey bees, predators and parasitoids (Lowery and Isman, 1995, Naumann and Isman, 1996, Walter, 1999). Our results indicated that Neemix applied at 11 and 45 ppm AZ did not significantly reduce the survival rates of *L. testaceipes* (Table 4). Although a small negative effect on parasitoid survival and emergence was observed in the 180 ppm treatment, this rate is probably not economically feasible and would not be required in applications for control of the brown citrus aphid. Neemix appears to be quite compatible with IPM in the citrus ecosystem. The overall results of this experiment are encouraging enough to warrant field trials of Neemix or other neem-based products for control of the brown citrus aphid.

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